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Growth Hormone Gene Polymorphism and its Association with Growth Parameters in Surti Goats

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A B S T R A C T

Surti is one of the well defined dual purpose (meat and milk) goat breeds of south Gujarat. Growth parameters are important for meat as well as milk production of the animals. Early selection of goats on the basis of growth parameters can fetch better economic returns to the farmers. Different molecular tools can help the early selection of the animals. PCR-RFLP is one of the basic techniques extensively used to study the genetic polymorphism in animals. The present study was aimed to study the genetic polymorphism in growth hormone gene (*GH*) and to find out its association with growth related parameters in Surti goats maintained at Livestock Research Centre, NAU, Navsari. DNA was extracted from the blood samples of 62 animals followed by the gene specific PCR. The digestion of *GH* PCR products with *HaeIII* enzyme revealed two genotypes (AB and BB). The genotypic frequencies of AB (422, 366 and 56 bps) and BB (366 and 56 bps) were found 0.8 and 0.2, respectively. The association analysis revealed that the animals with BB genotypes were having higher body weight ($P<0.05$) at six months of age as compared to the animals with AB genotypes.

Introduction

Goat is one of the main species as a source of income for landless and marginal farmers in India. The increase in goat population by more than 10 per cent in 20th livestock census as compared to 19th census indicates the goat as a preferred species for rearing by the farmers in our country (Anonymous, 2020). Goat contributes about 3 per cent in total milk production and 14 per cent in total meat production of India (Anonymous, 2019). Gujarat is home tract for important goat breeds like Zalawadi, Gohilwadi, Kutchi, Mehsani, Surti etc. Surti is one of the well defined goat breed of South Gujarat. The small sized breed is important for both milk as well as meat purpose. Growth parameters *viz.*, birth weight, body weight, heart girth, body length etc. are the good indicative

parameters to know the growth and ultimately the production performance in the animals. Growth hormone (*GH*) is a well identified candidate gene known to control either growth parameters as well as lactation yield traits in various livestock species including goats (Supakorn, 2009). Studies on the genetic variation in GH gene can help in identification of the cause of phenotypic variation. Genotyping the animals for this gene and establishing the association of the genotypes with the growth related economic traits can help breeders in early selection of animals and faster genetic improvement of the trait in the animals. PCR-RFLP is one of the basic and simplest molecular techniques used for genotyping the animals. Various studies have been reported for association of growth parameters with the genotypes of growth related genes in various goat breeds across the world including Indian goats using various molecular techniques (Hua *et al.,* 2009; Marques *et al.,* 2003; Gupta *et al.,* 2009; Ilham *et al.,* 2016; Kumar *et al.,* 2011; Zhang *et al.,* 2013; Silva *et al.,* 2012, Bayan *et al.,* 2018). Although, lot of progress has been achieved in animal improvement using conventional breeding methods, environmental influences limits accuracy of such methods for improving polygenic traits (Bin *et al.,* 2009) like body measurements (Singh *et al.,* 2015). Moreover, the information in Indian goats in particular Surti, is less. So the present study was planned with the objective to study the polymorphism in growth hormone gene using PCR-RFLP technique and to explore the association of various genotypes of the gene with the growth related parameters in Surti goats.

Materials and Methods

Five ml of blood was collected aseptically in EDTA vacutainers from jugular vein of 60 Surti goats of either sex maintained at Livestock Research Station, NAU, Navsari. The samples were stored at 4^{o} C till DNA extraction. The genomic DNA was extracted from whole blood by phenol-chloroform method as given by John *et al.,* (1991). Quality and quantity of the extracted DNA was ascertained by 0.8 % agarose gel electrophoresis and Nanodrop 2000c spectrophotometer, respectively. PCR was performed using gene specific custom synthesized (Europhins) previously reported primers F: 5' CTCTGCCTGCCCTGGACT 3' and R: 5' GGAGAAGCAGAAGGCAACC 3' to amplify exon 2 and 3 region of GH gene (Hua *et al.,* 2009). Total 20 µl of the reaction volume was prepared using EmeraldAmp® GT PCR Master mix (2x) 10.0 µl, forward and reverse primers (10 pmole/µl) each 0.6 µl, gDNA (30 ng/µl) 2.5 µl and MiliQ water 6.3 µl to make up the total reaction volume. PCR protocol was set in the Veriti thermal cycler (Thermo Fisher) step 1 as initial denaturation 94 ^{o}C for 10 min, followed by 30 cycles of step 2 as denaturation 94 ^{o}C for 1 min, annealing 62 ^{o}C for 1 min, extension 72 ^{o}C for 1 min and final extension as step 3 as 72 ^{o}C for 10 min. Five µl of PCR products were run on 2% agarose gel electrophoresis to check the amplification.

PCR-RFLP was carried out using restriction enzyme *HaeIII*. Total 20 µl of digestion mixture was set by adding 2.0 µl of 10x enzyme buffer, 0.4 µl of enzyme (10 U/µl) (NEB), 10 µl PCR product and MiliQ water to make up the volume. The digestion mixture was incubated at 37 ^{o}C for 15 min followed by inactivation at 80 ^{o}C for 20 min. PCR products digested with the restriction enzyme were run on 2% agarose gel electrophoresis to check the polymorphism.

The data pertaining to body weight, body length and heart girth of 1 week, 2 week, 3 week, 4 week, 2 months, 3 months and 6 months of age were collected for the goats under study. The statistical analysis to find

out association between the genotypes and mean values for growth parameters was carried out by comparing means using SPSS Statistics 20 package.

Results and Discussion

The good quality DNA samples with spectrophotometric 260/280 ratio between 1.8 – 2.0 and revealing compact single band on electrophoresis, were diluted to 30 ng/µl concentration followed by PCR amplification using above mentioned primers.

The product size of PCR for the gene was found to be 422 bps (Fig. 1). The digestion of GH PCR products with *HaeIII* enzyme revealed two genotypes i.e. AB and BB (Fig. 2). AB genotype was referred to the band pattern of 422, 366 and 56 bp while only 366 and 56 products were referred as BB genotype. The genotype and gene frequencies are presented in Table 1.

The results pertaining to association of genotypes with various growth parameters are depicted in Table 2. Genotype wise comparison of means through statistical analysis revealed that the animals with BB genotypes (366 and 56 bps) at 6 months were having significantly (P = 0.045) higher body weight then the animals possessing AB genotypes (422, 366 and 56 bps). The difference between means for the animals with different genotypes was non-significant for rest of the parameters.

Table.1 Genotypes and gene frequencies for the Growth Hormone gene in Surti goats

Gene (Total number of animals)	Genotypes	Observed genotypes	Observed genotypic frequencies	Observed gene frequency (A)	Observed gene frequency (B)
Growth Hormone (*GH*) (60)	AA	--	--	0.4	0.6
	AB	48	0.80		
	BB	12	0.20		

Table.2 GH gene genotype wise average of growth parameters at various ages in Surti goats

Parameter	Age of the animals	Genotype wise Mean ± SE		P value
		AB	BB	
Body weight (kg)	1 week	2.50 ± 0.13 (29)	2.43 ± 0.47 (7)	0.837
	2 week	3.38 ± 0.13 (32)	3.18 ± 0.31 (6)	0.557
	3 week	4.02 ± 0.16 (34)	3.83 ± 0.46 (6)	0.653
	4 week	5.25 ± 0.23 (41)	4.74 ± 0.56 (8)	0.369
	2 months	6.87 ± 0.22 (41)	6.89 ± 0.51 (9)	0.969
	3 months	8.44 ± 0.24 (43)	8.46 ± 0.60 (10)	0.968
	6 months	13.54 ± 0.28 (46)	15.18 ± 1.14 (11)	0.045*
Body length (cm)	1 week	31.21 ± 0.68 (28)	31.00 ± 2.08 (6)	0.903
	2 week	32.56 ± 0.55 (32)	34.00 ± 1.95 (6)	0.347
	3 week	34.76 ± 0.53 (34)	35.16 ± 2.18 (6)	0.796
	4 week	37.17 ± 0.57 (41)	36.5 ± 1.83 (8)	0.659
	2 months	40.66 ± 0.56 (41)	41.56 ± 0.80 (9)	0.479
	3 months	43.37 ± 0.54 (43)	44.30 ± 0.73 (10)	0.433

	6 months	50.50 ± 0.60 (46)	52.81 ± 1.33 (11)	0.100
Heart girth (cm)	1 week	31.36 ± 0.64 (28)	32.00 ± 1.86 (6)	0.693
	2 week	34.78 ± 0.59 (32)	34.67 ± 1.87 (6)	0.942
	3 week	36.38 ± 0.63 (34)	35.83 ± 1.49 (6)	0.737
	4 week	38.73 ± 0.58 (41)	38.13 ± 1.19 (8)	0.670
	2 months	41.98 ± 0.46 (41)	41.44 ± 0.91 (9)	0.627
	3 months	44.79 ± 0.46 (43)	44.70 ± 0.96 (10)	0.932
	6 months	51.72 ± 0.64 (46)	51.27 ± 1.05 (11)	0.755

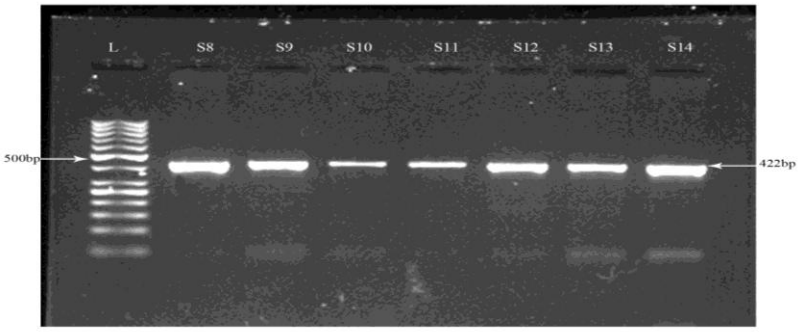

Figure 1: PCR Product of Growth Hormone (GH) gene of Surti Goats.
Lane: L-50bp Ladder, S8 to S14- PCR products of *GH* of Surti Goats.

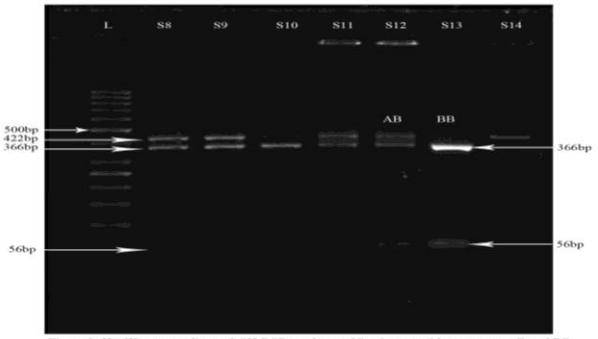

Figure 2: HaeIII enzyme digested GH PCR products of Sutri goats with genotypes AB and BB.
Lane: L-50bp Ladder, S8 to S14- PCR-RFLP products of *GH* of Surti Goats.

The frequency of AB genotype was found higher (0.8) with higher frequency for allele B (0.6). The population was not in Hardy-Weinberg equilibrium for this locus. Bayan *et al.,* (2018) have reported similar genotypes for the *GH* exon 2 and 3 regions in Surti as well as Mehsani goats. The polymorphism studies for the GH gene in Savanna and Kalahari goats by Amie Marini *et al.,* (2012) and in Egyptian goat breeds by Othman *et al.,* (2015) and Mahrous *et al.,* (2018) revealed similar genotypes with varying frequencies. The similar studies for *GH* in Kacang goats also revealed two genotypes (Ilham *et al.,* 2016). So many reports are in agreement with the findings of the present study. The population not being in Hardy-Weinberg equilibrium may be the result of selection practiced over generations on the farm. The absence of third genotype in most of the studies reveals the conserved gene sequence in the goat breeds across the countries.

Hua *et al.,* (2009) have found significant effect of genotypes for the same locus on weaning weight and chest girth at different age. Wickramaratne *et al.,* (2010) have reported significant effect of genotypes on

body weight and body length upto 9 months of age in Osmanabadi and Sangamneri goat breeds. Contrary to the present findings, Singh *et al.,* (2015) didn't find any difference in the growth parameters in the genotypic variants for the growth hormone gene.

In conclusion, *GH* has been found to be polymorphic for exon 2 and 3 region in Surti goats of Livestock Research Station, Navsari. The genotypes observed are found to be is associated with the phenotypic differences in the mean body weight of the kids at 6 months of age. So growth hormone gene can be considered as marker for early selection of Surti kids for body weight parameter. Though the results of the present study are in agreement with some of the previous reports, more studies are required in wider populations to establish the growth hormone polymorphism as a marker of choice for early selection.

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